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L1 9 METHYLOMONAS (8A) PHOSPHOFRUCTOKINASE

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L2 ANSWER 1 OF 8 CAPLUS COPYRIGHT 2005 ACS on STN

AN 2004:371066 CAPLUS

DN 140:369956

TI Natural promoters from Methylomonas genome for regulated gene expression  
in C1 metabolizing bacteria

IN Dicosimo, Deana J.; Picataggio, Stephen K.; Seip, John E.; Ye, Rick W.;  
Wang, Tao; Ni, Hao

PA E.I. Du Pont de Nemours and Company, USA

SO PCT Int. Appl., 83 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
	-----	---	-----	-----	-----
PI	WO 2004037998	A2	20040506	WO 2003-US33698	20031021
	WO 2004037998	A3	20040812		
	W: CA, JP, NO				
	RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR				
	US 2004126848	A1	20040701	US 2003-689200	20031020
PRAI	US 2002-419872P	P	20021021		

AB The invention relates to the use of promoter regions isolated from a  
Methylomonas sp. for gene expression and metabolic engineering in C1  
metabolizing bacteria. Genes, ntrA, glnB, htpG, moxP and hps, have been  
identified in the Methylomonas genome that are responsive to various  
metabolic and growth conditions. The identified responsiveness of these  
genes allows for the use of their promoters in regulated gene expression  
in transgenic C1 metabolizing bacteria. In particular, the hps promoter,  
which in its native state drives the expression of 3-hexulose-6-phosphate  
synthase (HPS), was found to be useful for directing expression of  
heterologous coding regions (e.g., crtZ) in the obligate methanotroph  
Methylomonas sp. 16a.

L2 ANSWER 2 OF 8 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

AN 2003:531666 BIOSIS

DN PREV200300533937

TI Genetic analysis of central carbon metabolism in the obligate methanotroph  
Methylomonas sp. strain 16a.

AU Sharpe, P. L. [Reprint Author]; Koffas, M. A. G.; Wilczek, J. [Reprint  
Author]; Knoke, K. L. [Reprint Author]; Odom, J. M. [Reprint Author]

CS DuPont, Wilmington, DE, USA

SO Abstracts of the General Meeting of the American Society for Microbiology, (2003) Vol. 103, pp. K-100. <http://www.asmtg.org/mtgsrc/generalmeeting.htm>. cd-rom.

Meeting Info.: 103rd American Society for Microbiology General Meeting. Washington, DC, USA. May 18-22, 2003. American Society for Microbiology. ISSN: 1060-2011 (ISSN print).

DT Conference; (Meeting)  
Conference; Abstract; (Meeting Abstract)

LA English

ED Entered STN: 12 Nov 2003

Last Updated on STN: 12 Nov 2003

AB Methanotrophs are unique in their ability to utilize methane (C-1 compounds) as a sole carbon and energy source. The utilization of C-1 compounds as carbon sources occurs via the assimilation of formaldehyde. Formaldehyde assimilation in methanotrophic eubacteria occurs via one of two pathways- the ribulose monophosphate cycle (RuMP) or the serine cycle. Variants of the RuMP cycle are able to utilize either the Enter-Doudoroff (ED) pathway or the Embden-Meyeroff-Parnas (EMP) pathway. The enzymatic activities of the EMP pathway enzymes are generally low in obligate methanotrophs. Therefore, obligate methanotrophs are thought to utilize the ED pathway exclusively. In contrast, facultative methylotrophs commonly utilize the EMP pathway. We have examined the pathway alternatives in the obligate methanotroph *Methylobacter* sp. 16a. We have evidence suggesting the possibility that both the ED and the EMP pathways are functional in *Methylobacter* sp. 16a (a RuMP cycle methanotroph). Whole genome sequence information indicates the presence of genes for both central carbon metabolism pathways. Growth and yield results from *Methylobacter* strains containing gene disruptions in genes coding for key enzymes in the ED pathway (fructose biphosphate aldolase) and the EMP pathway (2-keto-3-deoxy-6-phosphogluconate aldolase) demonstrate that both enzymes are important for the growth of *Methylobacter* sp. High pyrophosphate-linked **phosphofructokinase** enzymatic activities were also confirmed for this *Methylobacter* sp. Thus, in contrary to the existing literature, we have genetic and biochemical evidence that verifies that *Methylobacter* sp. strain 16a can utilize the EMP pathway for the assimilation of carbon.

L2 ANSWER 3 OF 8 CAPLUS COPYRIGHT 2005 ACS on STN

AN 2002:185333 CAPLUS

DN 136:242988

TI Sequences of *Methylobacter* genes and encoding proteins for exopolysaccharide biosynthesis

IN Koffas, Mattheos; Odom, James M.; Wang, Sigun; Wang, Tao; Ye, Rick W.

PA E. I. Du Pont de Nemours & Co., USA

SO PCT Int. Appl., 65 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002020797	A2	20020314	WO 2001-US26831	20010828
	WO 2002020797	A3	20020829		
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
	US 2002102697	A1	20020801	US 2001-934899	20010822
	US 6537786	B2	20030325		

CA 2417243	AA	20020314	CA 2001-2417243	20010828	
AU 2001086863	A5	20020322	AU 2001-86863	20010828	
EP 1358333	A2	20031105	EP 2001-966339	20010828	
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR					
US 2003148494	A1	20030807	US 2003-353457	20030129	
US 2003166171	A1	20030904	US 2003-353456	20030129	
NO 2003000962	A	20030409	NO 2003-962	20030228	
PRAI US 2000-229944P	P	20000901			
US 2001-934899	A3	20010822			
WO 2001-US26831	W	20010828			
AB	Nine Genes have been isolated from a <i>Methylobacter</i> sp encoding elements of the exopolysaccharide biosynthetic pathway. The genes and gene products are the first isolated from an organisms capable of utilizing single carbon (C1) substrates as energy sources. The genes are useful for engineering other C1 utilizing microorganisms to make altered levels of exopolysaccharide which is used in a variety of com. applications.				
L2	ANSWER 4 OF 8 CAPLUS COPYRIGHT 2005 ACS on STN				
AN	2002:185332 CAPLUS				
DN	136:242987				
TI	Methylobacter carbon metabolism pathway enzymes and genes and their use in alteration of carbon flow in methanotrophic bacteria				
IN	Koffas, Mattheos; Odom, James M.; Norton, Kelley Christine; Ye, Rick W.				
PA	E. I. Du Pont de Nemours & Co., USA				
SO	PCT Int. Appl., 73 pp.				
	CODEN: PIXXD2				
DT	Patent				
LA	English				
FAN.CNT 1					
	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
	-----	----	-----	-----	-----
PI	WO 2002020796	A2	20020314	WO 2001-US26730	20010828
	WO 2002020796	A3	20030123		
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
	US 2002110885	A1	20020815	US 2001-934901	20010822
	US 6555353	B2	20030429		
	CA 2416936	AA	20020314	CA 2001-2416936	20010828
	AU 2001085314	A5	20020322	AU 2001-85314	20010828
	EP 1313845	A2	20030528	EP 2001-964466	20010828
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
	US 2003129721	A1	20030710	US 2002-320924	20021217
	US 2003138909	A1	20030724	US 2002-320874	20021217
	US 6773905	B2	20040810		
	US 2004115657	A1	20040617	US 2002-321210	20021217
	US 6767744	B2	20040727		
	NO 2003000963	A	20030428	NO 2003-963	20030228
PRAI	US 2000-229906P	P	20000901		
	US 2001-934901	A3	20010822		
	WO 2001-US26730	W	20010828		
AB	Genes have been isolated from a <i>Methylobacter</i> 16a encoding enzymes in the carbon flux pathway. The genes encode both a 2-keto-3-deoxy-6-phosphogluconate aldolase (KDPGA) and a gene encoding fructose biphosphate aldolase (FFBPA) as well as numerous other genes. The gene will be useful in C1 metabolizing microorganisms for the manipulation of				

the carbon flux pathway.

L2 ANSWER 5 OF 8 CAPLUS COPYRIGHT 2005 ACS on STN  
AN 2002:185287 CAPLUS  
DN 136:242977  
TI Isoprenoid biosynthetic genes and enzymes from Methylomonas and their use  
for isoprenoid compounds production  
IN Cheng, Qiong; Koffas, Mattheos; Norton, Kelley C.; Odom, James M.;  
Picataggio, Stephen K.; Rouviere, Pierre E.; Schenzle, Andreas; Tomb,  
Jean-Francois  
PA E. I. Du Pont de Nemours & Co., USA  
SO PCT Int. Appl., 84 pp.  
CODEN: PIXXD2  
DT Patent  
LA English  
FAN.CNT 4

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002020733	A2	20020314	WO 2001-US26852	20010829
	WO 2002020733	A3	20030814		
	W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW			
	RW:	GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG			
	US 2002102690	A1	20020801	US 2001-934903	20010822
	US 6660507	B2	20031209		
	CA 2416940	AA	20020314	CA 2001-2416940	20010829
	AU 2001088476	A5	20020322	AU 2001-88476	20010829
	EP 1360301	A2	20031112	EP 2001-968214	20010829
	R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR			
	JP 2005500804	T2	20050113	JP 2002-525740	20010829
	NO 2003000830	A	20030430	NO 2003-830	20030221
	US 2004063143	A1	20040401	US 2003-700003	20031103
PRAI	US 2000-229907P	P	20000901		
	US 2001-934903	A3	20010822		
	WO 2001-US26852	W	20010829		

AB Genes have been isolated from Methylomonas 16a encoding the enzymes of isoprenoid biosynthetic pathway. The genes and gene products are the first isolated from a Methylomonas strain that is capable of utilizing single carbon (C1) substrates as energy sources. The nucleotide sequences and the encoded amino acid sequences of genes containing 9 ORFs encoding enzymes involved in isoprenoid biosynthesis are disclosed. The genes and gene products of the present invention may be used in a variety of ways for the production of isoprenoid compds. in a variety of organisms.

L2 ANSWER 6 OF 8 CAPLUS COPYRIGHT 2005 ACS on STN  
AN 2002:185282 CAPLUS  
DN 136:246501  
TI High growth methanotrophic bacterial strain  
IN Koffas, Mattheos; Odom, James M.; Schenzle, Andreas  
PA E. I. Du Pont de Nemours & Co., USA  
SO PCT Int. Appl., 157 pp.  
CODEN: PIXXD2  
DT Patent  
LA English  
FAN.CNT 4

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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PI	WO 2002020728	A2	20020314	WO 2001-US26827	20010828
	WO 2002020728	A3	20020711		
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
	US 2002137190	A1	20020926	US 2001-934868	20010822
	US 6689601	B2	20040210		
	CA 2416858	AA	20020314	CA 2001-2416858	20010828
	AU 2001086859	A5	20020322	AU 2001-86859	20010828
	EP 1320579	A2	20030625	EP 2001-966335	20010828
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
	JP 2004530403	T2	20041007	JP 2002-525735	20010828
	NO 2003000831	A	20030430	NO 2003-831	20030221
	US 2004147011	A1	20040729	US 2003-701200	20031104
PRAI	US 2000-229858P	P	20000901		
	US 2001-934868	A3	20010822		
	WO 2001-US26827	W	20010828		

AB A high growth methanotrophic bacterial strain capable of growth on a C1 carbon substrate has been isolated and characterized. The strain has the unique ability to utilize both methane and methanol as a sole carbon source and has been demonstrated to possess a functional Embden-Meyerhof carbon flux pathway. The possession of this pathway conveys an energetic advantage to the strain, making it particularly suitable as a production platform for the production of biomass from a C1 carbon source. Thus, the genetic and metabolic capabilities of *Methylobomonas* strain 16a were studied and defined.

L2 ANSWER 7 OF 8 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 1

AN 1993:145904 BIOSIS

DN PREV199395078704

TI Purification and properties of pyrophosphate-dependent  
**phosphofructokinase** of the obligate methanotrophic bacterium  
***Methylobomonas methanica***.

AU Beschastnyi, A. P.; Sokolov, A. P.; Khmelenina, V. N.; Trotsenko, Yu. A.

CS Inst. Biochem. Physiol. Microorg., Acad. Sci. Russ., Pushchino, Russia

SO Biokhimiya, (1992) Vol. 57, No. 8, pp. 1215-1221.

CODEN: BIOHAO. ISSN: 0320-9725.

DT Article

LA Russian

ED Entered STN: 16 Mar 1993

Last Updated on STN: 17 Mar 1993

AB Pyrophosphate-dependent phosphofructokinase (PP-1-fructose-6-phosphate: 1-phosphotransferase, EC 2.7.1.90) was isolated from the obligate methanotrophic bacterium *Methylobomonas methanica* 12 and purified to homogeneity. Data from PAAG electrophoresis in the presence and absence of sodium dodecyl sulfate suggest that the enzyme is a dimer with M-r = 92 kDa (2 times 45000). The pH optimum of the forward and reverse reactions is about 8.0. The apparent K-m values for fructose-6-phosphate, pyrophosphate, fructose-1,6-bisphosphate and orthophosphate are 0.38, 0.051, 0.1, and 1.7 mM, respectively. The enzyme is inactive in the absence of Mg-2+. The Mg-2+ concentration needed for the 50% activation of the enzyme in the forward reaction is 0.012 mM, that in the reverse reaction is 0.47 mM. The substrate specificity of the enzyme and the effects of metabolites on the enzyme activity were studied.



L2 ANSWER 8 OF 8 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN  
 AN 1989:249092 BIOSIS  
 DN PREV198987130157; BA87:130157  
 TI EFFECT OF GLUCOSE ON THE GROWTH AND METABOLISM OF OBLIGATE METHANOTROPHS.  
 AU SHISHKINA V N [Reprint author]; TROTSENKO YU A  
 CS INST BIOCHEM PHYSIOL MICROORG, ACAD SCI USSR, PUSHCHINO, USSR  
 SO Mikrobiologiya, (1988) Vol. 57, No. 6, pp. 917-923.  
 CODEN: MIKBA5. ISSN: 0026-3656.  
 DT Article  
 FS BA  
 LA RUSSIAN  
 ED Entered STN: 20 May 1989  
 Last Updated on STN: 28 Jun 1989  
 AB The ability of two obligate methanotrophic strains to assimilate glucose was studied. Glucose not only penetrated into *Methylomonas methanica* cells (type I), but also served as an additional carbon source (apart from methane) since about 17% of the carbon in the biomass originated from glucose. This methanotroph contained the following enzymes of carbohydrate metabolism: hexokinase, 6-phosphofructokinase, fructose-1,6-bisphosphate aldolase, glucose-6-phosphate and 6-phosphogluconate dehydrogenase, transaldolase, transketolase, ribulosephosphate-3-epimerase, phosphoriboisomerase, and 2-keto-3-deoxy-6-phosphogluconate aldolase. The metabolic blocks at the level of pyruvate kinase and  $\alpha$ -ketoglutarate dehydrogenase are presumed to account for the inability of *M. methanica* to grow on glucose. Glucose had no effect on the growth of *Methylosinus trichosporium* cells (type II) on methane because less than 1% of it contributed to the carbon of the biomass. This was due to the fact that the main pathways of carbohydrate metabolism were blocked in this methanotroph owing to the absence of hexokinase, 2-keto-3-deoxy-6-phosphogluconate aldolase, glucose-6-phosphate and 6-phosphogluconate dehydrogenases, and pyruvate kinase.